AD 12

(14) SUPDIV-7-76-Suppl

SUPPLEMENT

to

(6) PROCEEDINGS

1976

DIVERS' GAS PURITY SYMPOSIUM (3rd)

November 17 & 18, 1976,

~~Repository: [illegible]~~

Held at Battelle-Columbus.

Supplement.

(12) 23 p.

(11) 18 Nov 76

Sponsored by

U. S. NAVY SUPERVISOR OF DIVING
NAVAL SEA SYSTEMS COMMAND

Conducted Under Contract No. N00123-75-C-0377
Task No. 10

by

BATTELLE
Columbus Laboratories
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Columbus, Ohio 43201

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CARBON MONOXIDE-INDUCED ALTERATIONS IN PULMONARY ALVEOLAR MACROPHAGES AT AMBIENT AND ELEVATED PRESSURES

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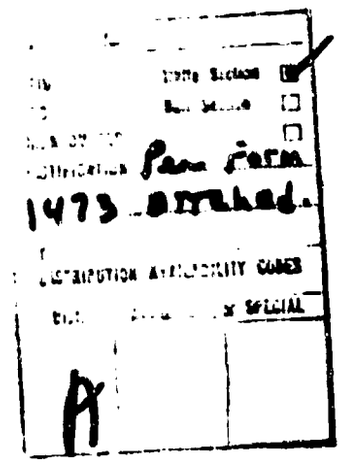

CARBON MONOXIDE-INDUCED ALTERATIONS IN PULMONARY ALVEOLAR MACROPHAGES AT AMBIENT AND ELEVATED PRESSURES[1]

ABSTRACT

The occurrence of carbon monoxide (CO) in diver's respirable gas supplies has been well documented. However, the sublethal effects of this asphyxiant in hyperbaric environments have not been adequately characterized. A physiologically sensitive indicator of sublethal effects of a contaminant is the pulmonary alveolar macrophage. The guinea pig alveolar macrophage and its reaction to various CO concentrations at 8 atmospheres absolute (ata) and their surface equivalent concentrations at 1 ata were studied in this experiment. In the absence of CO, macrophage counts and viabilities from guinea pigs exposed to a helium-oxygen environment at either 1 or 8 ata were not different from those of control animals in compressed air at 1 ata. The mean macrophage viability for guinea pigs exposed to CO concentrations at 1600-4200 mg/m^3 at 8 ata was 75.1 ± 6.3% and was not different from the 1 ata cell viability value of 72.0 ± 4.5% for the 1600-4200 mg/m^3 CO range. Five- to six-fold increases in total cell counts were observed with CO at both pressures. The data show that at 8 ata and CO concentrations of 1600-4200 mg/m^3, the decrease in alveolar macrophage viability was accompanied by a dramatic increase in alveolar macrophage counts.

[1]Supported by the Naval Medical Research and Development Command, NNMC, Department of the Navy, Research Task No. MPN10.01.1270B. The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the Naval Service at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council, DHEW Pub. No. (NIH) 74-23.

INTRODUCTION

Compressed gas supplies which are used for pressurization of chambers and submersibles and in life-support systems for divers have been chemically analyzed and found to contain carbon monoxide(1) in surface equivalent concentrations an order of magnitude greater than the threshold limit value of 55 milligrams per cubic meter (mg/m^3) promulgated by the Occupational Safety and Health Administration (2).

The U. S. Navy Bureau of Medicine and Surgery has established the limits for carbon monoxide (CO) in breathing air at 20 ppm (3). Since this level is not designated as a surface equivalent concentration (mg/m^3), it is important that experiments be conducted to evaluate the validity of this limit value in terms of a constant mass concentration independent of environmental pressure. The toxicological implications of carbon monoxide exposure are many. Carbon monoxide concentrations of 100-500 ppm at 1 ata elicit certain physiological adjustments, also, observed in chronic high-altitude hypoxia. Increases in hematocrit and hemoglobin concentration, increased tissue hypoxia, and cardiac enlargement are a few of the observed adjustments (4,5). A loss of motor performance and elevation of carboxy-hemoglobin concentration are also evidenced. Previous hyperbaric experiments have demonstrated that the LC50 for CO (lethal concentration of carbon monoxide

for 50% of the animals exposed) is 7038 ± 292 mg/m^3 and is not altered by pressures in the range of 1-8 ata when the CO concentration is expressed in mg/m^3 (6).

One of the living organism's major cellular defense mechanisms against a lower respiratory invasion of particulate matter is the pulmonary alveolar macrophage (PAM). The alveolar macrophage is a mononuclear cell which has long been known to participate in phagocytizing particulate matter (7). It is, therefore, regarded to be an important component of the pulmonary clearance mechanism. The phagocytized foreign matter is converted to harmless by-products by lysosomal enzymes in the macrophage. The macrophages and their entrapped foreign contents can either be absorbed into regional lymph nodes or removed by the mucociliary escalator system (7). The function of the PAM may either be affected by a toxic gas, such as CO, or by a combination of toxic gas and pressure.

One measurement indicative of the alveolar macrophage's functional state is the viability ratio, i.e., the percentage of living cells. The objectives of this study were to observe the comparative physiological effects of carbon monoxide on the PAM's of guinea pigs for surface equivalent concentrations at ambient and elevated pressures. The guinea pig was chosen as an experimental animal because toxicological studies indicate that this animal is much more sensitive to certain environmental

pollutants than are mice, rats, or rabbits (8).

METHOD OF EXPOSURE AND CONTAMINANT ANALYSIS

A 171 liter, 1000 psi chamber was used for all exposures. The oxygen partial pressure was maintained at 168 ± 10 mm Hg for the hyperbaric experiments with an oxygen detector (Teledyne 323 DP),[1] solenoid controller and a mass flow indicator (Tylan FMS-343).[2] During exposure of animals to He-O_2, the temperature of the chamber was maintained at 30° C for 1 ata and 31° C at 8 ata. These temperatures are comparable to ambient air at 24° C (9). It is necessary to increase the chamber temperature in a predominately helium environment because of the high thermal conductivity of a helium atmosphere. Carbon dioxide was removed from the chamber by using a Baralyme scrubber. Carbon monoxide concentrations were monitored at the chamber exhaust with a gas chromatograph (Tracor MT150)[3] equipped with an ultrasonic detector phase meter and a column switching valve for sample injection. Molecular sieve 5A was chosen for the column packing and a disc integrator was used for peak quantitation. An infra-red spectrophotometer (Miran 1)[4] provided an independent verifica-

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[1]Teledyne Analytical Instruments, San Gabriel, California

[2]Tylan Corporation, Torrance, California

[3]Tracor, Incorporated, Austin, Texas

[4]Wilks Scientific Corporation, South Norwalk, Connecticut

tion of contaminant levels. Three CO concentrations in the 1600-4200 mg/m^3 range were utilized for both 1 and 8 ata environments. Compressed air was used as the carrier gas in the 1 ata environment; whereas, helium-oxygen was used at 8 ata and for a control exposure at 1 ata. Guinea pigs were compressed with helium at a rate of 0.68 ata/min until the desired 8 ata depth was attained. The animals were allowed to stabilize in the helium environment at depth for one hour before the gaseous contaminant was bled into the chamber through a mass flowmeter and controller (Brooks Model 5811-12C2).[1] Each of the contaminant exposures was four hours from the time the desired concentration was obtained. A continuous decompression schedule, which adheres to the function P (psi) = $43e^{-.55t\ (minutes)} + 75e^{-.0452t}$, was derived from some previously conducted hyperbaric experiments in this laboratory and was utilized during these experiments. This function is valid only for 8 ata. This schedule did not introduce clinically visible signs of decompression sickness in guinea pigs and permitted complete decompression in 36 minutes.

EXPERIMENTAL ANIMALS AND TECHNIQUE FOR OBTAINING THE PULMONARY ALVEOLAR MACROPHAGES

The experiments were performed on male guinea pigs, NMRI:

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[1]Brooks Instrument Division, Emerson Electric Company, Hatfield, Pennsylvania

(HA)CV, with body weights of 285 ± 25 gms. They were immediately anesthetized with 30 mg/kg of sodium pentobarbital after the decompression schedule was completed. At least two of each six guinea pigs in an experimental group were used for the alveolar macrophage collection. A modification of Myrvik's technique for obtaining rabbit alveolar macrophages (10) is described in this report. The thoracic cavity was opened and the upper portion of the trachea was dissected free and clamped with a hemostat to exclude entry of blood into the lung after transection of the trachea. A cut was made above the point where the trachea was clamped (six tracheal rings above the carina) and the lungs, heart and trachea were dissected out as a unit. The excised tissues were washed with cold (4° C) 0.15 M sodium chloride with the trachea still closed to the atmosphere. The heart was carefully dissected free to avoid any injury to the bronchi and lungs. The lungs were sponged free of excess fluid, weighed and suspended by means of a hemostat clamped to the tracheal wall (lumen open). The sodium chloride solution was infused into the trachea through an inverted PE-100 cannula in the trachea until the lungs were slightly distended. This required approximately 8-12 ml of saline (5 ml/gm-lung tissue). The trachea was then occluded, while the lungs were gently massaged to obtain the macrophage-laden fluid. Five more washes were completed

in a similar fashion. Between washes the polyethylene cannula was clamped with a rubber-tipped hemostat to prevent loss of fluid or entry of air into the lungs. The cell suspensions were pooled and centrifuged for five minutes at 1085 x g. The supernatant was decanted and the cell button was resuspended in a quantity of cold physiological saline sufficient for a final volume of 5 ml.

Vital Cell Counts. The viability of the alveolar macrophages was determined by staining the cells with 1 ml of 0.1% trypan blue in borate buffer (pH 7.6). A sample of the cell suspension was placed under a coverslip on a Neubauer hemocytometer and examined with a microscope. The number of cells per cubic millimeter of cell suspension was determined according to the leukocyte counting method.

The count was multiplied by 10^3 to express the cell number in terms of cubic centimeter and by a factor of 12 to account for the final dilution of cell suspension with physiological saline and stain. The PAM counts are based on a gram-lung weight basis.

Macrophage protein concentration was determined by the Lowry method (11) using bovine serum albumin as a standard and a spectrophotometer (Gilford Model 300-N)[1] for absorbance

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[1]Gilford Instrument Laboratories, Incorporated, Oberlin, Ohio

measurements.

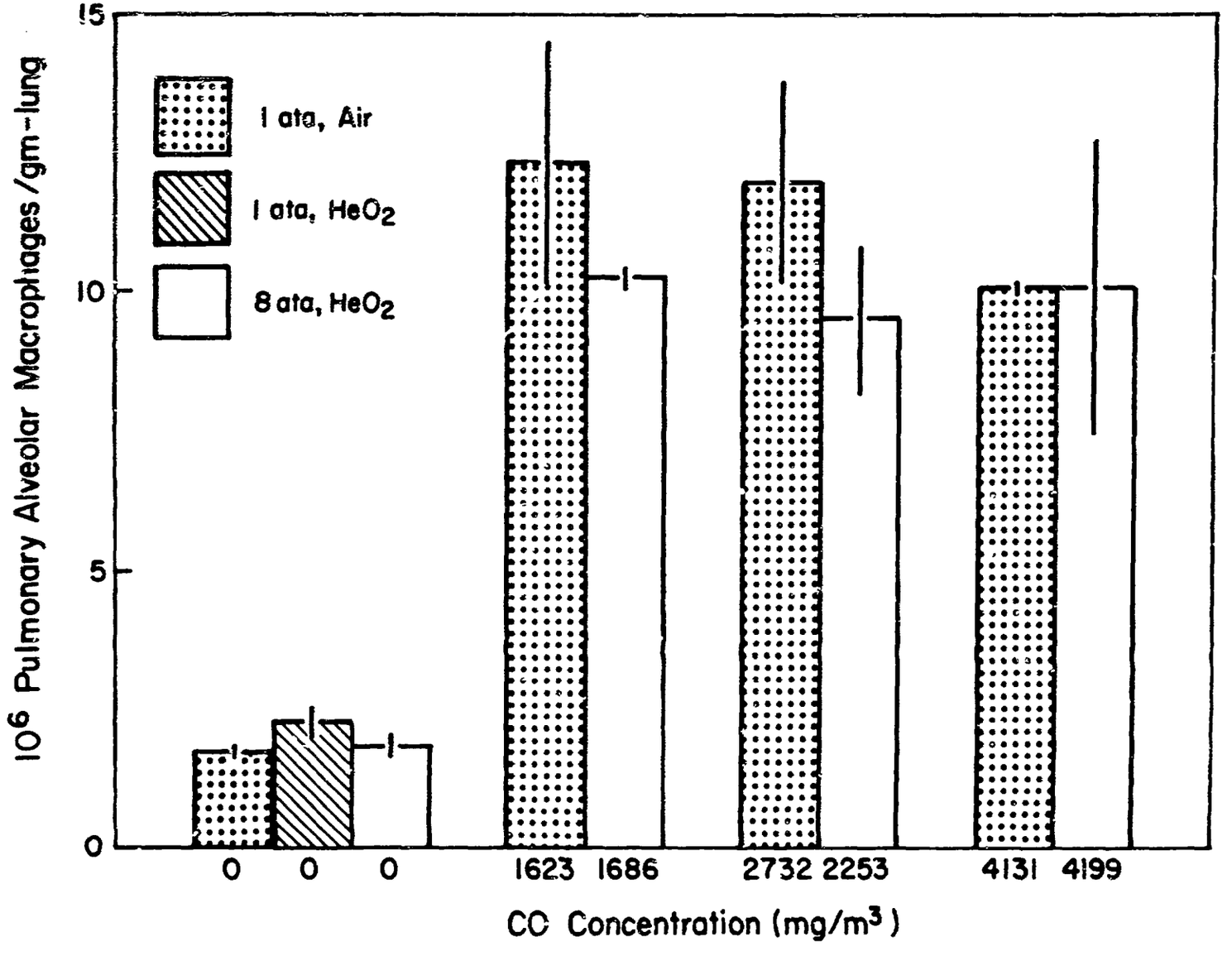
Results are expressed as mean values ± standard error. Analysis of variance was used to evaluate the extent to which the treatment and control animal groups differed from one another at each pressure. F-tests at a maximal level of 1.0% were conducted on these results. The Mann-Whitney rank-sum test and the sign test were used to evaluate the nonparametric data.

EXPERIMENTAL RESULTS

The number of alveolar macrophages obtained from lungs of guinea pigs exposed to compressed air was not different from those of guinea pigs exposed to He-O_2 at 1 ata or He-O_2 at 8 ata (Figure 1). The PAM counts obtained from animals not exposed to CO at 1 ata and those PAM counts from animals not exposed to CO at 8 ata were each compared to those groups of animals who were exposed to CO at either 1 or 8 ata. Significant differences exist among mean macrophage counts in the 1 ata treatment and control groups. The calculated F value was $P<0.01$.

Differential cell counts of macrophage pellets demonstrated 93% of the cells from control animals to be PAM's. A few randomly selected macrophage specimens from animals exposed to every possible combination of the CO concentrations and pressures demonstrated three- to six-fold increases

Figure I. CHANGES IN PULMONARY ALVEOLAR MACROPHAGE COUNTS IN MALE GUINEA PIGS EXPOSED TO VARIOUS CONCENTRATIONS OF CARBON MONOXIDE AT 1 AND 8 ATA.



of lymphocytes. An increased number of immature macrophages was observed.

Mean macrophage protein concentrations per gram-lung (micrograms macrophage protein/gm-lung) from guinea pigs exposed to any of the three CO concentrations at either 1 or 8 ata were different from those of controls (Figure 2). No real differences ($P<0.05$) could be observed between mean macrophage protein concentrations at 1 and 8 ata for any of the CO concentrations.

The survival rate (percent viability) of PAM's from guinea pigs exposed to He-O_2 at either 1 or 8 ata was not different from the macrophages of guinea pigs exposed to compressed air at 1 ata (Figure 3). Significant differences exist among mean PAM survival rates in the animals of 1 ata treatment and control groups. The calculated F value was $P<0.01$. Similar responses of decreased viability were observed throughout the CO concentration range, regardless of pressure. An overall mean for the macrophage viabilities at each of the CO concentrations at 1 ata was $72.0 \pm 4.5\%$. This survival rate is not different from the overall mean of $75.1 \pm 6.3\%$ calculated from macrophage viabilities of guinea pigs exposed at 8 ata to the various CO concentrations.

Protein concentrations per million alveolar macrophages from guinea pigs exposed to any of the three CO concentrations

Figure 2. CHANGES IN PULMONARY ALVEOLAR MACROPHAGE PROTEIN CONCENTRATION / GM-LUNG IN MALE GUINEA PIGS EXPOSED TO VARIOUS CONCENTRATIONS OF CARBON MONOXIDE AT 1 AND 8 ATA.

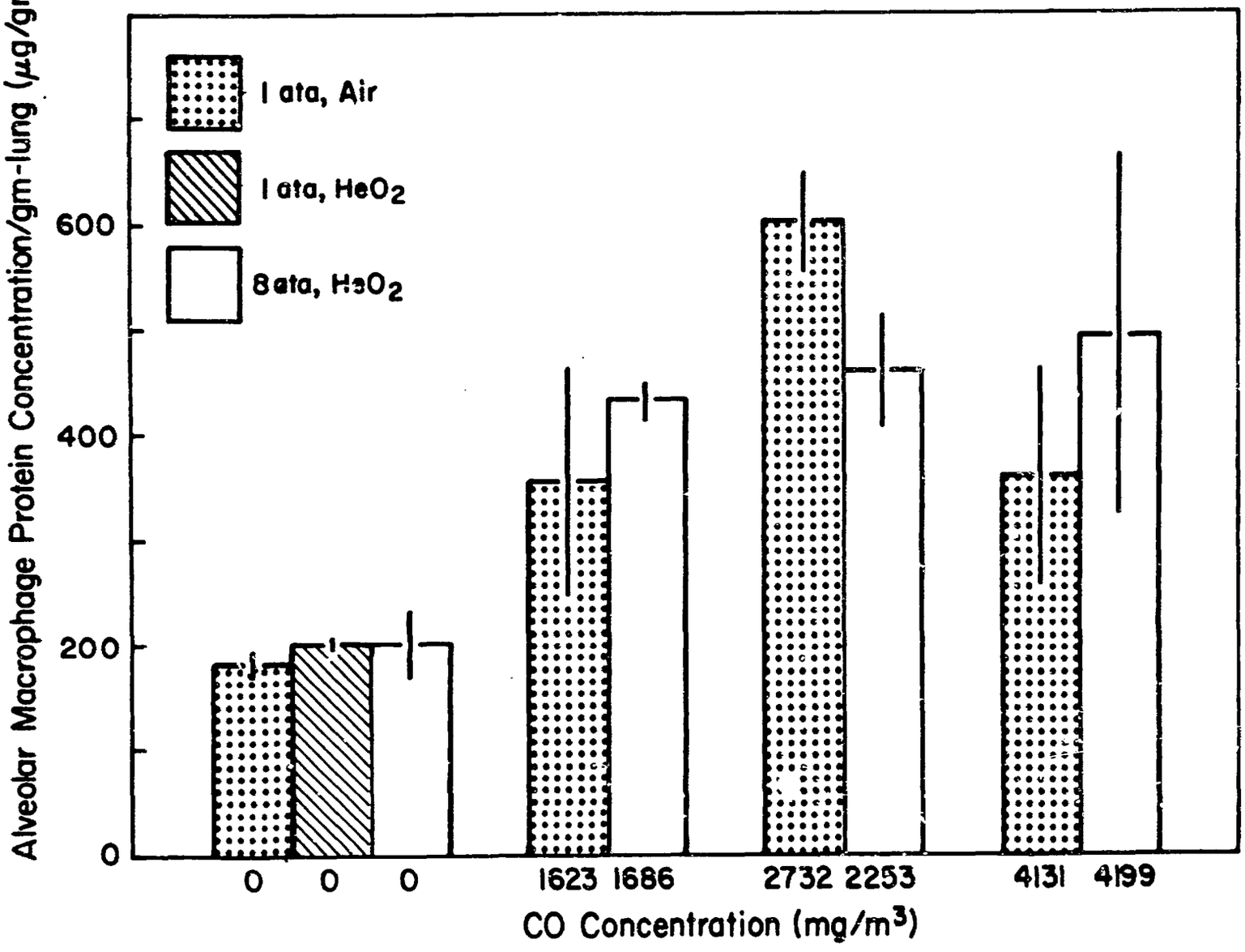
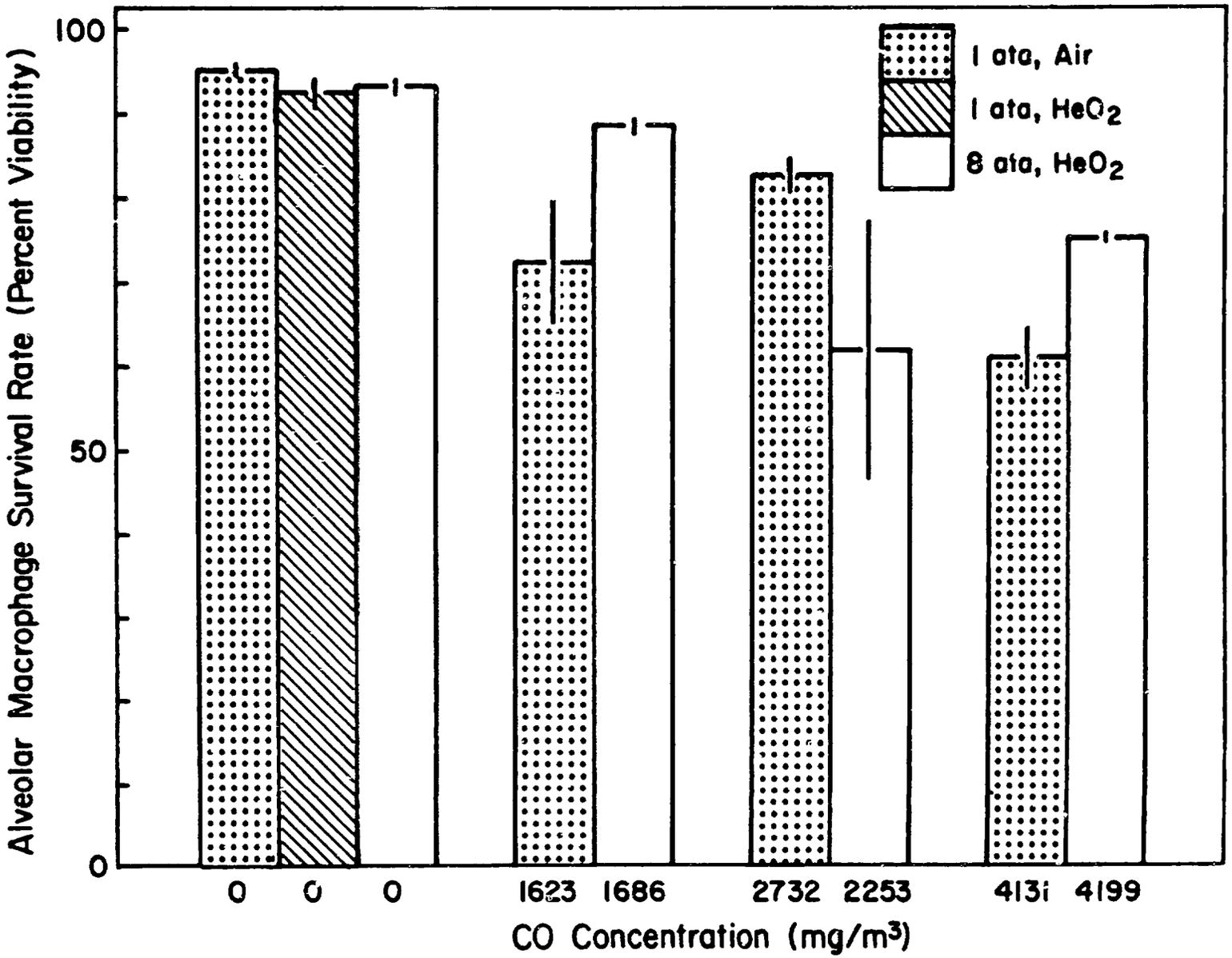


Figure 3. EFFECTS OF VARIOUS CARBON MONOXIDE CONCENTRATIONS AND INCREASED PRESSURE ON PULMONARY ALVEOLAR MACROPHAGE SURVIVAL IN MALE GUINEA PIGS.



at either 1 or 8 ata were significantly less than those of guinea pigs exposed at either pressure without CO (Figure 4). The calculated F value is $P<0.01$. Significant differences between protein concentration per million macrophages of guinea pigs exposed to 1 and 8 ata were only demonstrated at the lowest CO concentration. Excessive amounts of foamy material appeared with the pulmonary lavages of animals exposed to CO, irrespective of pressure. Approximately 1-2 mm of foamy material appeared in the macrophage suspensions from control animals. Two to five times as much foamy material appeared in macrophage suspensions from guinea pigs exposed to CO at either 1 or 8 ata.

DISCUSSION

The present investigation was an attempt to establish within a uniform exposure period of four hours the extent to which an inhalation exposure of sublethal concentrations of CO at 1 and 8 ata would alter the pulmonary alveolar macrophage in the guinea pig. A near equivalent number of carbon monoxide molecules (mg/m^3) was presented to the pulmonary alveoli of animals in either the 1 or 8 ata exposures.

The results of this experiment show dramatic five- to six-fold increases in alveolar macrophage counts for all three CO concentrations at either 1 or 8 ata. The survival rate of PAM's under equivalent concentrations of CO at 1 and 8 ata is

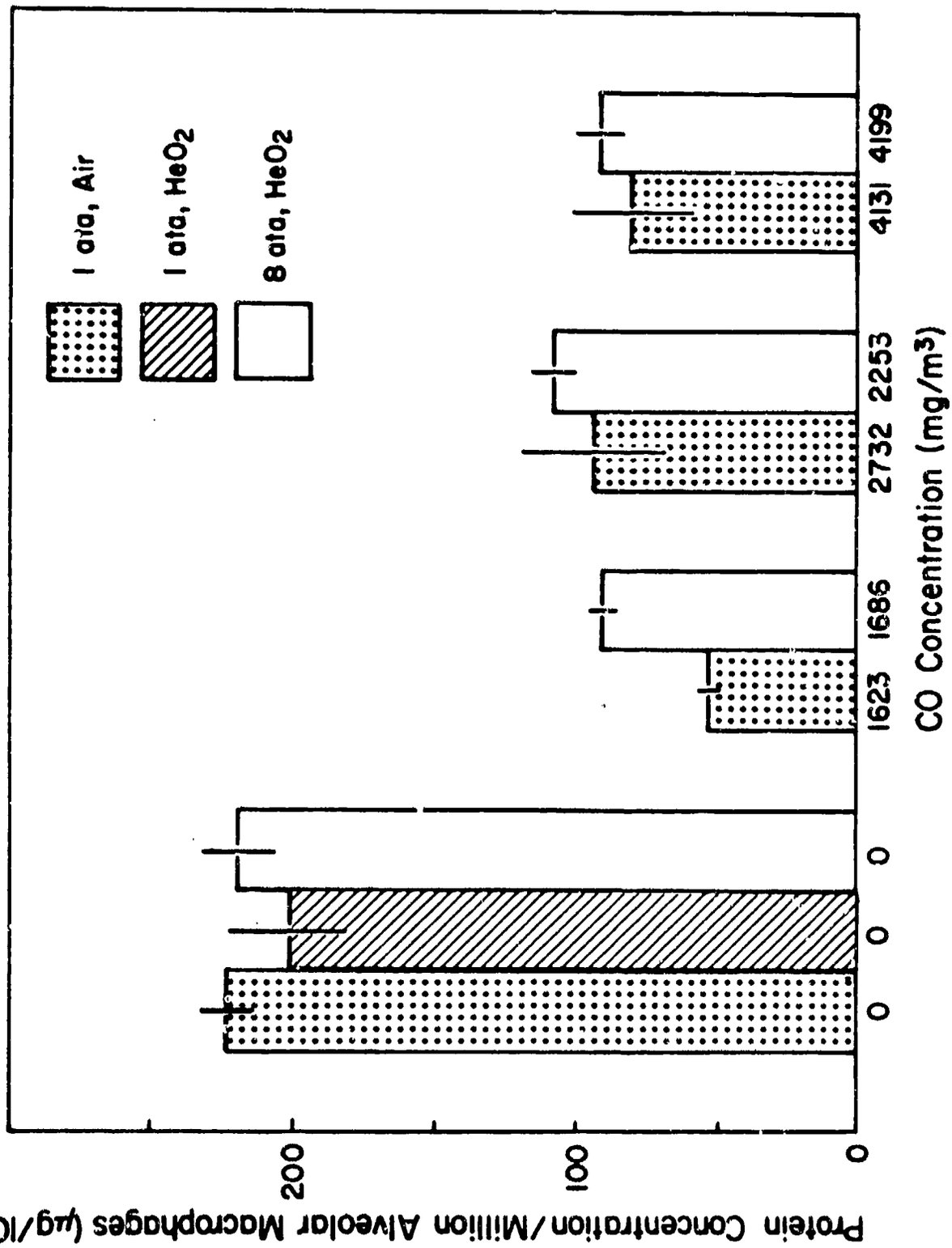


Figure 4. CHANGES IN PROTEIN CONCENTRATION/MILLION PULMONARY ALVEOLAR MACROPHAGES OF MALE GUINEA PIGS EXPOSED TO VARIOUS CONCENTRATIONS OF CARBON MONOXIDE AT 1 AND 8 ATA.

similar throughout the concentration range of 1600-4200 mg/m^3. The absence of differences in alveolar macrophage counts obtained from guinea pigs exposed to 1 and 8 ata at 0 mg/m^3 CO suggests that increased pressure does not affect the alveolar macrophage number. The similarity of increased PAM counts at each of the CO concentrations, regardless of pressure, would tend to support the idea that the increase in PAM's was not due to pressure. Though the asphyxiant did elicit phenomenal increases in PAM's over the 1600-4200 mg/m^3 CO concentrations, the cell survival rates were decreased.

Differentials on the randomly selected cell suspensions demonstrated that macrophages from lungs not exposed to CO at ambient or increased pressure were predominately mononuclear. A few polymorphonuclear cells were observed. Ninety-three percent of each cell suspension were macrophages. Similar differential cell types were observed on CO-exposed animals at either pressure. However, in several randomly selected cell suspensions from guinea pigs exposed to any of the three concentrations at either 1 or 8 ata, it was observed that there were three- to six-fold increases in lymphocytes. It was also observed that with the increase in lymphocytes in the macrophage counts of animals exposed to CO at either pressure there was a concomitant decrease in blood lymphocytes. Further studies are being conducted on this interesting phenomenon and

will be submitted for publication. The simultaneous decrease in lymphocytes of the blood and their increase in macrophage washes may demonstrate the manner in which the reticulo-endothelial system can augment the population of PAM's. It has been claimed that lymphocytes are capable of transformation to granulocytes, monocytes, macrophages, fibroblasts, epithelioid cells, giant cells, and plasma cells (12). It may be such a mechanism as this that caused the dramatic increases in PAM's of guinea pigs exposed to the various CO concentrations.

It is obvious from this experiment that the decreased viability was compensated for by the simultaneous increase in PAM's. The increased macrophage counts with 75% viability probably are more effective in pulmonary clearance than those control counts with 94% viability. Without this compensation the pulmonary clearance mechanism would be compromised in counteracting any lower respiratory invasion.

Increases in macrophage protein per gram-lung in guinea pigs exposed to any of the three concentrations of CO at 1 and 8 ata are due not to any increase in protein per macrophage but to the overall increase in cell counts. The increased number of immature macrophages and/or blood-borne macrophages would tend to dilute the protein concentration per macrophage because of the immature macrophage's smaller

cell size than that of mature macrophages.

The two- to five-fold increases in foamy material associated with washes from CO-treated guinea pig lungs may result from the increased number of macrophages recovered. Since alveolar macrophages possess surfactant on their cellular surface (13), it could be speculated that the increase in foamy material was due to a greater storage or transporting capability of PAM's for surfactant when the PAM's were exposed to CO.

SUMMARY

Macrophage number and viability have been demonstrated to be sensitive indicators of cellular response in guinea pigs exposed to CO at 1 and 8 ata. The increase in number of PAM's observed in animals exposed to the three concentrations of CO, independent of pressure, is important when considering the animal's ability to adapt to stress such as a response to remove foreign material from the respiratory system.

The finding in this study of decreased protein content per macrophage from animals exposed to various concentrations of CO, regardless of pressure, is in marked contrast to reported increases in protein content of macrophages exposed to cigarette smoke (14).

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

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1. REPORT NUMBER SUPPLEMENT SUPDIV No. 7-76	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SUPPLEMENT TO PROCEEDINGS 1976 DIVERS' GAS PURITY SYMPOSIUM		5. TYPE OF REPORT & PERIOD COVERED Proceedings, Nov 17 & 18, 1976 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s)		8. CONTRACT OR GRANT NUMBER(s) N00123-75-C-0377
9. PERFORMING ORGANIZATION NAME AND ADDRESS Battelle Columbus Laboratories 505 King Avenue Columbus, Ohio 43201		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Task No. 10
11. CONTROLLING OFFICE NAME AND ADDRESS Supervisor of Diving Naval Sea Systems Command Washington, D.C. 20362		12. REPORT DATE Nov 17 & 18, 1976 13. NUMBER OF PAGES 20
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

This is a one-paper supplement to the Proceedings

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

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20. (Continued)

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